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ASEXUAL BREEDING AND PREVENTION OF SENESCENCE IN PLANARIA VELATA.

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In an earlier paper¹ the asexual life-cycle of *Planaria* was described and it was shown that senescence leads to fragmentation of the larger part of the body, the fragments encysting and undergoing, like other isolated pieces, reconstitution into small whole animals which when they emerge from the cysts are physiologically young and capable of growth and repetition of the life-history. So far as known this is the only method of reproduction in the localities about Chicago where these worms are found, sexual reproduction never having been seen during some twelve years of observation.

I. ASEXUAL BREEDING.

In order to determine whether repeated asexual reproduction was possible without senescence of the stock the asexual breeding of the animals was begun in the spring of 1911 with a stock of animals collected from a temporary ditch in which the species was abundant. In the attempt to find suitable foods the stock was given varied food including lean beef, liver, kidney, earth-worm, fresh water mussel, crayfish, etc. In course of time it was found that the life-cycle could be considerably modified by different kinds of food and the results of the feeding experiments will be presented in another paper. After several months beef liver was found to be the most satisfactory food among those tested and has been the sole food of the stock since the early months of the experiment.

The worms of the stock collected in April, 1911, reached the end of their growth period, ceased to eat and began to fragment two to three weeks later and the cysts being kept in an abundant supply of fresh water during the summer, the young animals

¹ "The Asexual Cycle of *Planaria velata* in Relation to Senescence and Rejuvenescence," *Biol. Bull.*, Vol. XXV., No. 3, 1913.

emerged during September and October and after feeding grew rapidly and in about a month attained full size and began to fragment and encyst again. The worms of the third generation emerged from the cysts in three to four weeks and again attained full size in a little over a month and fragmentation and encystment began for the third time.

Up to the present time this cycle has continued to repeat itself more or less rapidly according to the frequency of feeding. During the last year the animals have been fed only three times a week instead of every day and growth has consequently been less rapid. For two months in the summer of 1913 the stock was placed in the refrigerator at a temperature of 8–10° C. in order to avoid possible loss from high temperature, and during one month of this time the animals were not fed. This procedure had, however, no other effect than to bring about fragmentation in a few individuals and a slight degree of reduction from starvation. The few fragments were discarded and in September the animals were brought back to room temperature, feeding was resumed and growth began again.

In this manner the stock has been carried through thirteen asexual generations in less than three years. At present (March, 1914) the thirteenth generation is approaching fragmentation and encystment.

The stock shows no indication of loss of vigor. The animals which emerge from the cysts in each generation show a high rate of metabolism and are physiologically young and undergo senescence in each generation in the same way as the animals in nature. During the period of breeding there has been no indication of the development of sexual organs so far as could be determined by external examination. Certainly no genital openings have ever developed and no eggs have been laid.

As compared with the almost four thousand asexual generations of *Paramecium* bred by Woodruff the number of generations attained by this stock is small but the fact that a metazoan species is capable of passing through twelve asexual generations without any indications of sexual reproduction is of interest.

Moreover, it is not because the animals do not become old that they are capable of continued asexual reproduction. It was

shown in the paper on the asexual cycle of this species referred to above that senescence is manifestly associated with growth in each generation and that the occurrence of asexual reproduction in this species is a result of the decrease in rate of metabolism which occurs in the course of senescence. In that paper it was also shown that asexual reproduction, which is essentially a process of isolation of pieces and their reconstitution into whole small individuals, brings about rejuvenescence as a result of the reorganization and reduction which occur in the process of reconstitution. Evidently the animals undergo a regression to a comparatively early stage of development with each reconstitution and it is also evident that the degree of rejuvenescence in each reproduction is on the average the same, for the stock does not as yet show any indication of a progressive senescence from generation to generation.

Whether gradual, progressive senescence of the stock is entirely eliminated by the process of reproduction or will sooner or later become apparent with continued asexual breeding can of course be determined only by further breeding of the stock and it is the writer's intention to continue the experiment as long as seems necessary. But the apparent absence of sexual reproduction in this species under natural conditions, to which attention was called in the earlier paper, constitutes strong evidence for the conclusion that the species is able to maintain itself indefinitely by asexual reproduction alone. Since it is demonstrated that asexual reproduction brings about rejuvenescence in this species as well as in *Planaria dorocephala* there is no apparent reason why asexual reproduction should not continue indefinitely without senescence of the stock or race. All that is necessary for the realization of this possibility is that the degree of rejuvenescence in each generation should be on the average the same and that is apparently the case.

II. REJUVENESCENCE BY STARVATION.

Planaria velata like *P. dorocephala* and other species of *Planaria* undergoes reduction in size when starved and this reduction may be continued until the animal is but a small fraction of its original size. The reduction in size is of course

due to the fact that the animal uses up its own tissues as a source of energy and since it contains no skeleton which takes little or no part in reduction a very great decrease in size may occur before death.

In a recent paper¹ it was shown that in *Planaria dorotocephala* the susceptibility of the animals and also the rate of CO₂ production increases as the animals undergo reduction from starvation. These changes certainly indicate an increase in rate of metabolism during starvation and reduction. The only difference between the animals reduced in size by a long period of starvation and young growing animals is that the starved animals possess almost no capacity for acclimation to low concentrations of KCN, alcohol, etc., while the young growing animals possess a high capacity for acclimation. When the starved animals are again fed this difference disappears almost at once and they are in all respects physiologically young and are capable of renewed growth and of repeating the life cycle.

In *Planaria velata* the same increase in susceptibility occurs during starvation as in *P. dorotocephala*, the reduced animals show almost as high a susceptibility as young growing animals of the same size and when feeding is resumed this species is also capable of renewed growth and of once more going through the life cycle. The susceptibility determinations give essentially the same results as in starved individuals of *P. dorotocephala*. Estimations of CO₂ production and determinations of capacity for acclimation have not been made for starved individuals of *P. velata* because it seemed unnecessary in view of the other facts.

There is then no doubt that starvation and reduction bring about rejuvenescence in *P. velata* as in *P. dorotocephala*. The large old worms with low susceptibility before starvation are in essentially the same physiological condition after starvation and reduction followed by renewed feeding as young growing animals of the same size and are capable of repeating the life history from the stage at which feeding is resumed.

¹ Child, C. M., "Starvation Rejuvenescence and Acclimation in *Planaria dorotocephala*," *Arch. f. Entwicklungsmech.*, XXXVIII, 3, 1914.

III. INHIBITION OF SENESCENCE BY PARTIAL STARVATION.

If complete starvation and the resulting reduction bring about rejuvenescence it should be possible by feeding animals enough to prevent reduction but not enough to permit growth to keep them indefinitely in practically the same physiological condition and so to prevent senescence.

In 1911 a part of the stock used for asexual breeding was isolated in the second asexual generation after collection and the attempt was made to feed this stock only enough to maintain the worms at approximately the same size. During the early part of this experiment too much food was given and a few of the animals underwent partial fragmentation and encystment. All such individuals were removed from the stock and the experiment was continued with the remainder, these being completely starved for several weeks after fragmentations occurred in the stock in order to reduce their size and bring them back into a physiologically younger condition in which fragmentation would not occur. Since the early part of the experiment, the food has been somewhat further reduced in quantity and no further fragmentations have occurred. The stock was small at the beginning consisting of some forty worms. Some of these were lost by the early fragmentations and since that time animals have occasionally crept out of the water and dried upon the sides of the dish and others have been lost by being removed on the pieces of food or have been accidentally poured out in changing water but there have been no deaths or losses from other than these accidental causes. The stock now consists of five animals. As regards feeding the procedure finally adopted and still adhered to is to feed two or three times at intervals of two days, and as soon as the animals begin to increase in size to stop feeding for two or three weeks or until they are reduced to their former size. In this way the animals have been kept during most of the two years between four and seven millimeters in length. Whenever individuals of the stock show more rapid growth or reduction than the others they are isolated and fed or starved until they are of the same size as the others when they are again returned to the stock. During most of the time the stock has been fed with pieces of earthworm, because experience has shown that

with this food the animals in general attain a larger size before ceasing to feed and undergoing fragmentation than when fed with liver. But when earthworm is given in sufficient quantities senescence occurs, though its course is somewhat different from that of senescence with liver as food. The effects of different foods on the course of the life cycle will be discussed at another time.

During the months of July, August and September of 1913 the stock was kept in a refrigerator at a temperature of about 10° C. in order to avoid the danger of encystment from high temperature, and feeding during this period was of course less frequent since the animals were less active and required less food to maintain a constant size. During September they were not fed at all and underwent reduction to a somewhat greater extent than usual in the starvation periods. At the beginning of October they were brought back to room temperature and since that time the feeding has been continued as before.

The five worms which now make up the stock are in the same generation as they were two years ago and of somewhat smaller size, about five millimeters, than at the beginning of the experiment. They are active, behave like young animals, react very strongly to food and appear in every respect to be as young physiologically as growing worms of the same size. Unfortunately the small size of the stock has not permitted determinations of susceptibility at intervals, but judging from the activity and appearance of the animals their susceptibility would be that of young animals. While these animals have remained in the same generation during more than two years and are to all appearances as young physiologically as at the beginning, in fact somewhat younger, since they are kept at a smaller size than when first isolated as a stock, the other portion of the same generation which was used for asexual breeding has in the same length of time passed through twelve asexual generations with the cycle of high susceptibility, growth with decreasing susceptibility, cessation of feeding, fragmentation, encystment, reconstitution in the cysts and emergence in each generation. In these partially starved animals the changes characteristic of the life cycle have been inhibited and they have remained at practically the same

stage while other members of the same original generation have given rise to twelve generations of descendants. As a matter of fact the animals have not actually remained at exactly the same stage during this time but their life has consisted of alternating progressions and regressions of slight extent as periods of feeding and starvation have alternated.

IV. CONCLUSION.

This partial starvation experiment affords an interesting contrast to the experiment in asexual breeding. The latter demonstrates that the animals may undergo senescence and rejuvenescence for generation after generation of asexual reproduction without any indications as yet of progressive senescence of the stock. The former, on the other hand, demonstrates that senescence may be prevented by partial starvation for at least a length of time equal to twelve generations and judging from the present indications both experiments may be continued indefinitely, although the partial starvation experiment will finally be terminated by accidental losses, since there is of course no increase in the number of animals such as occurs in the breeding experiment.

Senescence in these animals is evidently associated with growth and rejuvenescence with reduction and reconstitution and neither has any necessary relation to sexual reproduction. In papers referred to above the writer has advanced the view that senescence in its simplest terms consists in a decrease in rate of metabolism resulting from the proportional decrease in amount of the metabolic substratum and from changes in the substratum which retard to the chemical reactions of metabolism. Rejuvenescence on the other hand is an increase in rate of metabolism resulting from the removal of inactive or less active substance and from changes in the substratum which permit a higher rate of reaction. Growth and differentiation bring about senescence and reduction and reconstitution bring about rejuvenescence. The facts of the present paper constitute further evidence in support of this view and show not only that the rejuvenescence of old animals is possible without reproduction of any kind but also that senescence can be prevented or at least retarded so as to be inappreciable

for a long period of time by preventing growth and the later stages of differentiation.

SUMMARY.

1. *Planaria velata* has been bred asexually through thirteen generations in less than three years without any indications of progressive senescence in the stock. In each generation the animals have passed through the following cycle: reconstitution and rejuvenescence in the cysts, emergence as physiologically young, small animals, growth and senescence with feeding, cessation of feeding, fragmentation and encystment. During the period of breeding none of the animals have ever become sexually mature.

2. Starvation and reduction being about an increase in rate of metabolism and the reduced animals after renewed feeding are in the same physiological condition as young growing animals and are again capable of growth and senescence.

3. Senescence has been inhibited or so far retarded as to be inappreciable in a stock of *Planaria velata* during more than two years by partial starvation. During this time the animals have been kept at approximately the same size and in approximately the same physiological condition, viz., that of half grown animals, and no reproduction has occurred. During the same period another stock of animals collected at the same time and originally in the same generation has been fed and bred asexually and has passed through twelve generations.

4. In this species neither sexual nor asexual reproduction is necessary for the production of young individuals from old. Senescence is associated with growth and differentiation and rejuvenescence with reduction and reconstitution. In the asexual cycle senescence and rejuvenescence alternate and apparently balance each other, at least during thirteen generations and probably will continue to do so indefinitely.

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